PEPTIC AND TRYPTIC DIGESTION PRODUCTS AS INEXPENSIVE CULTURE MEDIUMS FOR ROUTINE BACTERIOLOGIC WORK

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In the course of our studies on the carrier problem in enteric infections and our inquiries into the nature and distribution of dysentery infections on the Pacific Coast, we were compelled to investigate the reliability of the various culture mediums which are used. The need of large quantities of mediums made it necessary to find proper or superior substitutes for the reliable, but expensive, veal infusion-peptone-broth. In comparing the merits and values of the numerous vegetable or animal substitutes for "peptone," which have been recommended since the beginning of the war, we found certain peptic and tryptic digests to be excellent substrata for the usual types of culture mediums. These digests are inexpensive, readily prepared in any laboratory, and should therefore be used in conserving the food supply of a country at war.

From the observations of Bainbridge[1] and the studies of Sperry and Rettger,[2] and Rettger, Berman and Sturges,[3] it is evident that the amino-acids and other nitrogenous substances which readily give up their nitrogen as a result of bacterial action, are particularly responsible for the food value of culture mediums. It is a common observation that "Chapoteaut" and certain American peptone solutions give more luxuriant growth of bacteria than an identically prepared solution of Witte's "peptone." Chemical analyses have demonstrated a higher amino-acid content in the "Chapoteaut" (20%) than in American "peptones." Moreover, Duval,[2] Hottinger[5] and others have called attention to the fact that in the usual process of making beef or veal broth the greater part of the nutritive elements of the meat is lost.

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[1] Jour. Hyg., 1911, 11, p. 341.
[2] Jour. Biol. Chem., 1915, 20, p. 445.
[3] Jour. Bacteriol., 1916, 1, p. 15.
[4] Jour. Med. Research, 1913, 28, p. 165.
[5] Centralbl. f. Bakteriol., I, O., 1912-1913, 67, p. 178.

Indeed, it is not unlikely that in numerous instances very little food material suitable for the pathogenic micro-organisms is extracted when antiquated, uncontrolled methods are used. Therefore, it is not surprising that, as a result of careful comparative studies, Hottinger[5] recommended a process of slow digestion with "pancreatin." Wastefulness is furthermore avoided by this method, as sufficient peptone is obtained by the tryptic digestion to obviate the addition of commercial "peptone." With the exception of the studies of Dalimier and Lancereaux on the value of "opsine" (a digestive product resulting from the combined action of pepsin, trypsin and erepsin on a mixture of proteins) and the recent report of Davis,[7] little attention has been paid in this country to the use of digestion products as culture mediums. Undoubtedly the scarcity of Witte's peptone in England accounted for the investigations of Douglas,[8] Cole and Onslow,[9] which will be considered more carefully in the latter part of this paper.

On the other hand, the failure to obtain strong diphtheria-toxin with peptones other than "Witte's" have prompted several investigators—particularly Robinson and Rettger,[10] and Davis[7]—to ascertain whether the animo-acids, peptones, or some of the various types of proteoses are essential for the development of the toxin. The report of Robinson and Rettger suggested that probably proteoses of polypeptids, resultants of the peptic digestion, are essential to the formation of the toxin. This view is quite in harmony with the fact that Martin's peptone broth and Witte's "peptone" (which as far as present information is available are both peptic-digestion-products) are mediums in which very strong toxins are produced as a rule.

Recent reports by D. J. Lloyd,[11] Cole and Lloyd,[12] and others indicate that highly parasitic micro-organisms, like the meningococcus and gonococcus, require for primary cultivation in vitro certain accessory growth factors (vitamins or growth hormones). The amount of these vitamins necessary to stimulate growth is apparently dependent on the amino-acids present in the medium. From the studies of these workers it is quite evident that the main food requirements of the meningococcus and gonococcus are the products obtained by the hydrolysis of

[6] Arch. de méd. expér. et d'anat. path., 1913, 25, p. 449.
[7] Jour. Lab. and Clin. Med., 1917, 3, p. 75.
[8] Lancet, 1914, 2, p. 891.
[9] Ibid., 1916, 1, p. 9.
[10] Jour. Med. Research, 1917, 134, p. 357.
[11] Jour. Path. and Bacteriol., 1916, 21, p. 113.
[12] Ibid., 1917, 21, p. 267.

proteins. From this brief review it is apparent that the use of peptic or tryptic digestion products is fully justified.

Most of the commercial peptones supply a moderate amino-acid content at a very high cost. On the other hand, tryptic digestion products (of casein, for example) prepared with remarkable ease in any ordinary bacteriologic laboratory, furnish these substances in large quantities and in comparatively constant amounts.

We have, therefore, during the past year studied some of the practical methods recommended for the manufacture of amino-acids and used the same for the preparation of routine mediums. The present report is preliminary to further researches. It is perhaps well to emphasize the point that our main efforts thus far have been concentrated on applying known procedures to utilize animal protein which has either been disregarded as a food source, or has been treated in such a manner that most of the nutritive value has been lost.

The methods employed by us are fully considered in the appendix. The final broth preparations were analyzed chemically[13] to obtain some comparative figures of the most important elements. These data are briefly summarized in Table 1. Only digestion products which repeatedly gave results identical with or superior to the ordinary veal-broth substratum are mentioned. A diversity of micro-organisms, some with very selective food requirements, were tested repeatedly on different lots of digest-mediums.

The following digestion products have thus far been investigated: (1) Tryptic digestion of human placenta. (2) Peptic and tryptic digestion of beef- and sheep-blood. (3) Peptic and tryptic digestion of pig- or beef-liver. (4) Tryptic digestion of beef-heart and "pancreatin medium," according to Hottinger. (5) Tryptic digestion of casein, "tryptamine broth," according to the method of Cole and Onslow. (6) Autolysis of pigs' and dogs' livers, based on the studies of Bradley.

1. *Tryptic Digestion of Human Placenta.*—In bacteriologic laboratories of hospitals the use of placentas as culture mediums can be highly recommended. Simple placental infusions which are used instead of beef or veal are not economical even when they improve the growth of meningococci [(Kutscher)

[13] The determinations were made by means of the following methods:

Total Nitrogen: Kjeldahl's method. Non-Protein Nitrogen: Protein precipitated with trichloracetic acid-Kjeldahl's method on filtrate. Amino-Nitrogen: Van Slyke's method (Jour. Biol. Chem., 1913, 16, p. 121). Chlorids: McLean and Van Slyke's method (Jour. Biol. Chem., 1915, 21, p. 361). Inorganic Phosphates: Marriat's and F. H. Haessler's method (Jour. Biol. Chem., 1917, 32, p. 233).

TABLE 1

RESULTS OF EXPERIMENTS IN THE COMPARATIVE STUDY OF VARIOUS NUTRIENT MEDIUMS

Medium	Total Nitrogen per 1,000 C C	Protein Nitrogen per 1,000 C C	Non-protein Nitrogen per 1,000 C C	Percentage of Protein Nitrogen of Total Nitrogen	Percentage of Amino-nitrogen of Total Nitrogen	Total Solids per 1,000 C C	Ash	Chlorids per 1,000 C C	Phosphates per 1,000 C C	Cost per 5,000 C C of Finished Agar
	Grams	Grams	Grams			Grams	Grams	Grams	Grams	
1. Veal infusion—Witte's peptone broth	3.472	.826	2.646	23.8	10.2-10.6[2]	23.988	4.0885	3.25	0.2	About $1.62[1]
2. Veal infusion—"Difco" peptone broth	3.248	.35	2.898	10.7	13.8	20.924	4.812	....	.....	About $1.74
3. Trypsinized bullock heart according to Douglas	3.08	.956	2.124	31.2	18.5	25.049	3.08	3.32	0.212	76 cents-$1.00 depending on the trypsin used for the process
4. Peptic digest used by the authors:	3.668	....	....	....	14.7	....	....	....	....	About 43 cents-50 cents
a. Lean beef digest.....	3.108	....	....	....	.....	....	....	....	....	
b. Pig liver digest.....	4.76	1.106	3.654	23.2	17.0-21.0	29.406	7.216	3.75	0.125	24-30 cents[3]
Several samples digested for 24 hours	3.164	....	....	....	.....	....	....	....	....	
Several samples digested for 48 hours	3.544	....	....	....	.....	....	....	....	....	
Several samples digested for 72 hours	4.620	....	....	....	16.6-17.0	....	....	....	....	
c. Trypsinized pig liver digest 24 hours at 37 C.	3.696	.538	3.158	14.7	35	....	....	....	....	30-33 cents
d. Beef blood clot digest 24 hours	4.13	1.31	2.82	32.2	18	....	....	3.62	0.0930	21-25 cents
5. "Aminoids" (5%) broth....	6.244	....	....	....	28.5	23.08	4.1978	....	....	$1.20
6. Tryptic digest of casein according to Cole and Onslow	3.906	1.143	2.763	29.1	37.0	....	....	....	....	25-40 cents depending on the trypsin used for the process
7. Pancreatin-broth accordng to Hottinger	7.440	....	....	....	26.0	....	....	....	....	55 cents
8. Whole blood broth according to Szasz and Schmitz	....	....	....	....	.....	....	....	....	....	52 cents
9. Liebig's or Lemco extract broth	....	....	....	....	.....	....	....	....	....	58 cents
10. Potato broth.....	....	....	....	....	.....	....	....	....	....	25 cents

[1] This price includes waste of the veal.
[2] These figures represent averages obtained from several determinations.
[3] This price does not include the expenditure for electric current nor the initial investment for the digester.

or acid fast organisms (Duval and Wellmann)]. Such infusions contain so small amounts of amino-acids (1.5%, Slemons) that they require the addition of commercial "peptones" and mineral elements.

One placenta is sufficient to prepare 2-2.5 liters of broth. Tryptic digestion with ordinary pancreatic extract or trypsin liquor, according to Method 2 of the appendix, gave liquid and solid mediums in every respect similar to the tryptic-broth of Douglas. These cheap digests, when adjusted to a slightly alkaline reaction (P_H 7.3-7.4) and mixed with defibrinated human- or rabbit's-blood, or hydrocele fluid, are particularly suited for the primary isolation of micro-organisms of the respiratory tract and the gram-negative diplococci of the meningo-gonococcus group. Sterilized placental digests keep well when stored at room temperature.

2. *Peptic or Tryptic Digests of Whole Blood or Blood Clots.*—As a substitute for meat, whole blood has been recommended by Szasz,[14] Schmitz[15] and others; their claims have been supported by Kelser[16] who considers the mediums prepared from whole blood superior in many respects to the ordinary laboratory preparations. Our investigations fully confirmed this point, but we were not satisfied with the method of using blood as a substratum, because expensive peptone preparations have to be added to the infusion of the clot and serum. The excellent results noted with the various peptic and tryptic digests of beef-heart or pigs' liver suggested the same procedure for the blood infusion. The methods which proved satisfactory are given under Method III of the appendix.

From Table 1 it will be noted that this blood digest "contains a large percentage of amino-acids" and is exceedingly cheap. The use of this medium in serum institutes, where blood clots are constantly available, can be highly recommended. We have used it as an equivalent substitute for other digests and found it to be excellent for the growth of delicate-growing organisms, like streptococci. In combining the procedure of Schmitz[15] or of Lloyd[11] by adding "vitamines" in form of defibrinated blood to the finished agar, or by clearing the agar with beef serum, a medium resulted which proved of dependable quality for the primary isolation of pneumococci and meningococci. In this form the medium can practically take the place of blood or serum-agar prepared in the ordinary manner.

3. *Peptic and Tryptic Digestion of Pigs' or Beef-Liver.*—Martin's broth, so highly recommended by French workers and others, was proven in our hands the only medium adapted for the isolation of filtrable viruses, particularly the pleuropneumonia virus. Some preliminary tests convinced us that an extraordinary medium for general laboratory work can be procured when, in the preparation of the peptone, there is also included some veal, beef, rabbit muscle, or—as Martin[17] has suggested—some liver pulp. For economic reasons we abandoned the use of beef, but experimented with rabbit muscle which was always available, and we found the resulting medium to be superior to the ordinary veal-broth. Our main interest, however, centered on the applicability of the liver digests. Remarkable results were obtained in our typhoid studies, so that we can fully recommend this digest not only as a substitute for veal-broth or agar, but also as a substratum which gives results superior to those obtained

[14] Centralbl. f. Bakteriol., I, O., 1915, 75, 489 and 77, p. 111.
[15] Ibid., 1916, 76, p. 306.
[16] Jour. Bacteriol., 1916, 1, p. 615.
[17] Compt. rend. Soc. de Biol., 1915, 78, p. 261.

on the usual mediums recommended for the isolation of typhoid and dysentery organisms from the stool. The method of preparing these mediums is fully described in the appendix, Method I.

With the exception of the adjustment of the reaction, no particular difficulties will be encountered in the making of the basic digest broth. Phenolphthalein titration has proven, as usual, to be unreliable, and we recommend either the customary adjustment of the substratum with litmus paper or one of the colorimetric methods used for the determination of the H-ion concentration of biologic fluids. We used the method of Hurwitz, Meyer and Ostenberg[18] in accordance with the suggestions of Clark.[19] The simple method of Cole and Onslow[9] gives equally dependable results. It has been our custom to adjust a total bulk of 10 liters to a reaction of the P_H 7.0, and make the final adjustment in accordance with the special cultural requirements of the organisms for which a batch of medium is used.

A chemical analysis of several different lots of peptic liver digests (25 liters) is shown in Table 1. It will be noted that the percentage of amino-nitrogen, determined by the "Van Slyke method" varies between 17 and 21%. These slight differences are in part due to the filtration of the broth which was not always conducted in the same manner. Similar differences were noted with various lots of veal broth containing the same amount of Witte's peptone from the same powder. In using 5 and more pigs' stomachs and keeping the other factors constant, digests are regularly obtained of a remarkably even composition, the amino-nitrogen representing 18% of the total nitrogen.

In comparison with the veal peptone broth, these mediums contain from four-fifths to twice the amount of amino-acids, as in Witte's peptone broth, together with proteoses which are present in about the same proportion.[20] The amino-acid content of the peptic digest is the same as determined for the trypsinized broth according to Douglas. The total solids and the ash are also increased in comparison with the infusion-peptone-broth.

The only deficiency is found in the phosphate content, which is readily remedied by adding 0.2-0.4% dibasic potassium phosphate (K_2HPO_4) or diammonium phosphate. Henderson[21] and recently Kligler[22] have repeatedly called attention to the extreme value of mixtures of basic and acid phosphate salts in regulating bacterial metabolism and in acting with the proteoses as buffers in culture mediums. A culture fluid, rich in amino-acids but poor in phosphates, will give rapid initial growth which will soon cease, however, on account of the appreciable changes that small increases in acid or alkali have on the H-ion concentration.

Aside from the amino-acid content the presence of carbohydrates is noteworthy. Some samples were fermented with fresh broth cultures of B. coli or of B. saccharolyte; from 10-25% gas was produced inside of 12-16 hours. The presence of these carbohydrates is in part responsible for the excellent growth of the primary cultures of the colon-typhoid group, a point which we shall consider in another paper. For the stock cultures, however, this high carbohydrate content (particularly in the absence of phosphates or similar "buffers")

[18] Bull. Johns Hopkins Hosp., 1916, 27, p. 16.

[19] Jour. Bacteriol., 1917, 2, p. 211.

[20] Two estimations of the proteoses were made by precipitation with saturated zinc sulphate solution.

[21] Jour. Med. Research, 1917, 16, p. 1.

[22] Jour. Bacteriol., 1917, 2, p. 351.

TABLE 2

RESULTS OBTAINED WHEN DEFINITE MIXTURES OF PURE BROTH CULTURES OF B. COLI, B. TYPHOSUS AND B. PARADYSENTERIAE WERE USED

Twenty-Four Hours Old Broth Cultures Plated on:		B. Coli (Stool) Million Organisms per C C in an Average of 5 Plates			B. Typhosus Million Organisms per C C in an Average of 5 Plates			B. Paradysenteriae (Hiss-Y-Russell) Million Organisms per C C in an Average of 5 Plates		
		Veal Agar	Peptic Digest Agar	Pancreatin Agar	Veal Agar	Peptic Digest Agar	Pancreatin Agar	Veal Agar	Peptic Digest Agar	Pancreatin Agar
Veal infusion—Witte's peptone broth P_H 7.2	1st Exper.	260	240	210	230	210	260	80	90	120
	2nd Exper.	230	240	240	190	200	180	280	270	250
Peptic digest broth P_H 7.2	1st Exper.	420	450	460	330	360	390	230	250	330
	2nd Exper.	430	460	440	310	350	340	220	240	281
Pancreatin broth P_H 7.2	1st Exper.	170	230	170	190	150	160	40	80	50
	2nd Exper.	150	170	170	180	190	180	50	70	70

TABLE 3

BROTH DILUTIONS OF 1: 1,000,000, 1: 10,000,000 AND 1:100,000,000 PLATED IN 10 C C OF PEPTIC DIGEST AGAR (P_H 7.4)

Organisms Grown in the Following Mediums:	B. Coli Million Organisms per C C			B. Typhosus Million Organisms per C C			B. Paratyphosus Million Organisms per C C			Shiga B. Dysenteriae Million Organisms per C C			(Hiss-Y-Russell) B. Paradysenteriae Million Organisms per C C		
	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours	24 hours	48 hours	72 hours
Veal infusion — Witte's broth P_H 7.4.....	270	>300	450	10	155	205	180	157	192	80	>300	106	170	187	225
Second Exper. { P_H 7.5	333	...	...	243	...	...	...	...	...	...	...	...	...	...	...
Second Exper. { P_H 7.0	231	...	...	138	...	...	...	...	...	...	...	...	...	...	...
Peptic digest broth P_H 7.4.....	280	>300	592	30	263	554	240	183	225	130	>300	180	260	272	241
Second Exper. { P_H 7.5	337	...	...	419	...	...	...	...	...	...	...	...	...	...	...
Second Exper. { P_H 7.0	121	...	...	171	...	...	...	...	...	...	...	...	...	...	...
Pancreatin broth (rabbit) P_H 7.4.....	220	470	302	20	70	57	120	125	89	10	4	97	100	73	201

is a disadvantage and should be corrected by the addition of phosphate salts and calcium carbonate. For this purpose it is better, however, to use sugar free digests only.

Most of the broth preparations give a distinct bile salt reaction; in our laboratory digests with strong reactions are particularly reserved for typhoid work.

For the primary isolation of pneumococci, streptococci and like organisms, a higher amino-acid content and known glucose content are sometimes desirable. To accomplish this result, we trypsinized the neutralized sterile digest with 1% trypsin solution for 18-24 hours at 37 C., and simultaneously removed the carbohydrates by adding 0.5% of a 24-hour-old broth culture of B. saccharolyte. Subsequent analyses demonstrated (Table 1) an increase of amino-nitrogen to 35% of the total nitrogen. The final broth contains more suitable protein split products for bacterial growth than the beef broth prepared by slow digestion with "pancreatin" of Hottinger. In comparison with the casein digest prepared according to the method of Cole and Onslow, approximately the same amount of amino-acids is obtained in a considerably shorter time interval.

The progress of the hydrolysis can readily be followed by testing the tryptophane content[23] of the digest in intervals of 12-24 hours. As a rule the peptic digests of liver, blood or beef give only a faint reaction; properly trypsinized mediums, on addition of bromin water, always produce a deep reddish-violet color reaction, the pigment being soluble in amyl alcohol.

According to our experiments, prolonged action of trypsin is inadvisable because the nutritive value is rarely enhanced when the peptic digest is treated for more than 24 hours. The neutralized, trypsinized broth is mixed to advantage with 0.1% dextrose, C.P., 0.2% K_2HPO_4, and 1% calcium carbonate. Agar mediums prepared from the trypsinized liver digest are cleared in our laboratory by the addition of beef or sheep blood (D. Lloyd's "vitamine" agar), and have repeatedly proven to be the most dependable type of culture medium for the isolation of highly parasitic organisms. Our experience of a few months permits us also to conclude that the trypsinized sugar-free liver digest mediums, to which calcium carbonate and phosphate have been added, are well suited for stock cultures of pneumococci, streptococci and meningococci. For various reasons already outlined, peptic digests cannot be recommended for this purpose.

For plating, peptic and tryptic digests can be diluted with tap water from one-half to two-thirds of their volume, or preferably with 0.6% saline or a dilute solution of meat extract. Most of the ordinary micro-organisms grow well on these diluted digests. On the saline diluted agar the number of colonies are frequently increased in comparison with plates made from undiluted digestor from veal infusion-agar.

The only objection to the regular use of the diluted digests is found in a tendency to the development of small colonies, particularly for our routine work, diluted mediums did not show any advantage over the ordinary standard preparations. Progressive dilution of the digests—as Table 1 readily explains—will reduce the food value of the medium and probably introduce unknown factors with regard to the salt and "buffer" content, which we cannot consider until further studies have been completed.

In Tables 2 and 3 some of the numerous bacteriologic tests, which were carried out with various digests, are enumerated for the purpose of comparing their value with those obtained by the Standard methods. The results are

[23] For quantitative estimation use the method given by Levene and Rouiller, Jour. Biol. Chem., 1907, 2, p. 481.

illustrative of the already stated fact that peptic digests as a rule give a better initial as well as a more prolonged growth of the typhoid-colon group, than "pancreatin media" prepared according to Hottinger or the ordinary veal infusion peptone mediums. The superiority of the peptic digests over "pancreatin media" is conclusively shown in the tables.

We have repeatedly noted that the growth of bacteria on peptic digests is twice as heavy as on the ordinary standard mediums; the observation is particularly valuable in the preparation of bacterial vaccines or large amounts of bacterio-proteins. Peptic liver digests are well suited for indol tests and have also given reliable results as mediums for the detection of gas and acid formation.

Experiments with comparatively small quantities (4-8 liters) of peptic digests demonstrate the possibility of producing a concentrated "extract-like" preparation, which can be handled in a manner similar to Liebig's beef extract. Through the use of heat and a vacuum, concentrated extracts are obtainable, which can be reduced on the water-bath to gummy, brownish, hygroscopic masses. Repeated tests with several small samples proved in every respect identical with the original digest. We are continuing these experiments with the aim of producing peptic digest in easily transportable form, adapted to field and war conditions.

(4) *Tryptic Digestion of Beef Heart* (Douglas) *and the "Pancreatin medium"* (Hottinger).—A series of tests has been carried out with the peptone broth which is obtained by the method of Douglas. This particular medium has attracted considerable attention, being a part of the pea-flour extract medium recommended by Gordon and Flake[24] for the primary isolation of meningococci and by Lloyd for the preparation of stock cultures of these organisms. We can fully support the claims of Douglas: the results obtained with the medium are excellent in every respect. In its original form, or modified for our particular needs, the substratum has a wide range of application. We have used it particularly in Loeffler's serum and egg medium. To encourage the use of this medium, we give in the appendix a detailed outline of the method of preparation.

According to Table 1, the chemical analysis of trypsinized beef heart showed an amino-nitrogen content of 18%, the total nitrogen being somewhat less than are the peptic liver digests. Chlorids and phosphates are present in proper amounts. The great advantage of this tryptic digest over the slow peptic digests consists in the rapidity with which a medium with a high amino-acid content can be prepared. The time to manufacture a good culture medium is considerably shorter than we have found necessary for all the other types of mediums we have investigated. In our opinion, the only objection is the cost. Independent of the price of trypsin, the initial cost of the bullocks' hearts will always double the price of the finished agar in comparison with the peptic liver digest which supplies the same amount of amino-acid by a somewhat more tedious process. On the other hand, beef hearts are less expensive than veal, and we hope that the time is not far distant when the latter will be entirely replaced by the more economical heart muscle.

The "pancreatin medium," made according to the method described by Hottinger, does not offer sufficient advantage over the other mediums, already discussed, to compensate for the trouble involved in its preparation. The "stock-broth," in a dilution of 1 . 10, gives an agar substratum which is inferior to the standard meat-extract-peptone-medium. Many strains of pathogenic bacteria (streptococci, dysentery) on account of the high amino-acid content (Table 1,

[24] Brit. Med. Jour., 1916, 2, p. 678.

7) gave abundant initial growth, but could not be cultivated successfully for any length of time; most of the strains died out after a few transplants. Tables 2 and 3 also illustrate the fact, that "pancreatin" broth or agar is not equal to veal infusion peptone mediums and is inferior to peptic digests of liver or blood.

In substituting rabbit's muscle (which is available in our laboratory from the cadavers of the experimental animals) for beef, we have prepared by the Hottinger process a medium which in a dilution of 1:20 was not only inexpensive but was also of sufficiently constant composition that it could be used by students and beginners in practicing the fundamental bacteriologic technic.

(5) *Casein Digest Prepared According to the Method of Cole and Onslow.* —In previous paragraphs we have referred to the value of tryptic digests. The inexpensive and effective substitute for peptone which was prepared by Cole and Onslow by digesting commercial casein, belongs to this group of digestion products and is therefore included in the scope of our experiments.

We have used a cheap granulated casein which is obtainable in the local market (California creameries) and which is fairly uniform in composition; it is free from carbohydrates and alkalies. Digestion was accomplished with pancreatic extract prepared from pigs' pancreas, or with "Bacto" trypsin.[25] The original method of Cole and Onslow was otherwise carefully followed.

One sample of diluted casein broth, chemically analyzed, gave an amino-nitrogen content of 37%, the highest amount of amino-acid in any finished culture medium thus far studied by us. Other samples, titrated by the Sörensen method and therefore not included in Table 1, gave similar readings. Repeated comparative tests with various organisms, milk and water counts, support the claims of Cole and Onslow that "tryptamine" broth, or agar, supplies at exceedingly low cost a very high concentration of amino-acid in a faintly colored solution which is of fairly uniform composition, provided the same casein product is employed in the process of digestion. In our experience the only disadvantages are the prolonged digestion necessary to obtain a good product and the correspondingly large number of flasks which have to be incubated to insure a regular supply of mediums.

For primary isolation of organisms of the typhoid-dysentery, pneumococci, meningococci group, the peptic liver digests, trypsinized beef heart, or placenta mediums have given better growth than the casein preparations. Some substances, the nature of which is at present unknown, are apparently lacking in the casein digests.[26] Repeated tests have shown that the addition of veal or beef heart infusion (in the proportion of 2 parts infusion to 1 part of casein) give a substratum which is superior to the simple "tryptamine stock broth." Further experiments—already begun by us—are expected to throw some light on these important points and will be published, if they prove to be of any value. For the present we feel justified, however, in recommending the "tryptamine broth" for general application in the study of carbohydrate fermentations and similar bacteriologic procedures (toxin production of spore bearing anaerobes) in which a broth with an abundance of amino-acids, approaching more nearly a synthetic medium, is desirable.

(6) *Liver Autolysates Prepared in Accordance with the Studies of Bradley.* —The recently published studies of Bradley[27] and his collaborators suggested the

[25] Prepared by Digestive Ferments Company, Detroit, Mich., U. S. A.

[26] In this connection we desire to call attention to the observations of Burrows and Neymann (Jour. Exp. Med., 1917, 25, p. 93), that tissue cells are unable to live in the presence of any great concentration of α-amino-acids. It is not unlikely that certain of these acids in high concentrations disturb also profoundly the metabolism of highly organized bacteria.

[27] Jour. Biol. Chem., 1915, 21, 209; 1915, 22, 113; 1916, 25, 261.

use of dogs' or pigs' liver autolysates in N/50 normal hydrochloric acid (1 part of liver to 5 parts of acid) preserved by the addition of chloroform and toluene. After 15-20 days' incubation at 37 C., the deep-brownish autolysates are neutralized with sodium carbonate heated for 30 minutes at 100 C., and filtered. The filtrate diluted in the proportion of 1 part to 20 parts of 0.5% saline. Amino-acid determinations by the Sörensen method gave, on the average, 10-15% amino-nitrogen in the diluted broth. The growth of the test organisms was satisfactory and corresponded well with that obtained with "pancreatin media." Our experiments with these autolysates antedated those with peptic digests and, until quite recently, remained preliminary. The development of a practical method, further detailed chemical analyses, and other important points are still under investigation, and we hope to publish the results in the very near future.

SUMMARY

Inexpensive culture mediums for routine bacteriologic work can be readily prepared from peptic or tryptic digestion products. On account of the high amino-acid content these substrata furnish preparations superior to the usual standard mediums and are of sufficiently constant composition to warrant their general use.

APPENDIX

I. PREPARATION OF PEPTIC DIGEST BROTH OF LIVER, OF BEEF OR OF HUMAN PLACENTA

1. Wash, clean and mince finely 5 or more large pigs' stomachs. Mince an equal amount of clean pigs' or beef liver, cheap fat-free beef, placenta or blood clots.

2. Mix in the following proportions:

Minced stomachs	400 gm.
Minced liver, beef or placenta	400 gm.
Hydrochloric acid (Baker Chemical Co.)	40 gm.
Tapwater at 50 C	4,000 gm.

and keep the mixture at 50 C. for 18-24 hours.[1]

3. Make a biuret[2] and also a tryptophan test.[3] When both reactions are positive, the digest has a yellowish-greenish color and contains very little undigested debris.

4. Transfer to large bottles[4] and steam for 10 minutes at 100 C. to stop digestion. Strain the digest through cotton, or preferably store over night in the ice-chest and decant after 24 hours.

[1] It is advisable to digest the mixture in glass or porcelain receptacles. We have been accustomed to placing museum jars with the digest in a large, electrically regulated water-bath. Very good results, however, have also been obtained by using a large enameled pot equipped with a "Therm-Elect" thermo-regulator and heating unit installed by the Electric Sales Service Company of San Francisco.

[2] Five c c of filtered digest, add 0.1 c c of 5% solution of copper sulphate; mix and then add 5 c c normal sodium hydroxid. Pink color indicates complete peptonization.

[3] To 10 c c of neutralized and filtered digest add slowly bromin water until the maximum purple coloration is reached.

[4] The bottles in which the Baker Chemical Company supply hydrochloric or sulphuric acid are excellent for this purpose.

5. Warm the filtrate or decanted digest to 70 C. and neutralize with sodium carbonate (twice normal solution) to litmus at this temperature.

6. Sterilize (if not to be used at once) in the autoclave at 10 lbs. pressure for 15 minutes, or for 30 minutes at 100 C., on 2 successive days and store away.[5]

This stock digest is used for the various mediums as follows:

(*a*) *Plain Digest-Broth:*

1. Filter the desired amount into a flask.

2. Add 0.2% dibasic potassium phosphate (K_2HPO_4).

3. Set the desired reaction by using litmus, or preferably to a definite H-ion concentration (P_H 7.0-7.5) by one of the reliable methods recommended for the determination of the H-ion concentration. (Hurwitz, Meyer, Ostenberg, Clark and Lubs, or Cole and Onslow.)

4. Heat the broth in the steamer at 100 C. for 15 minutes.

5. Correct the reaction and filter through paper.

6. Distribute in the receptacles used for cultures and sterilize at 100 C. according to the usual routine method.

(*b*) *Sugar-Free Digest-Broth:*

1. Inoculate the sterile "stock" digest in a flask or bottle with 1% of a 24-hour old broth culture of B. saccharolyte or B. coli and incubate for 12-18 hours at 37 C.

2. Steam for 20 minutes.

3. Adjust the reaction and add 0.2-0.4% dibasic potassium phosphate and 2% purified talcum. Filter through paper, distribute for use, sterilize; or use the turbid, killed saccharolyte culture (without previous cleaning) for the preparation of agar (note method given under "*c*").

(*c*) *Stock Digest Agar:*

1. Take a measured quantity (8-10 liters) of stock digest; add 0.2% dibasic potassium phosphate (K_2HPO_4), and 2% of agar fiber.[6]

2. Autoclave at 10 lbs. pressure for three-quarters of an hour or heat in a double-boiler to 100 C. and keep the mixture at this temperature until the agar is dissolved.

3. Restore the volume lost by evaporation.

4. Set the reaction very lightly alkaline to litmus or to P_H 7.3 by using twice normal NaOH or KOH. Special attention should be given to the adjustment of the reaction, because some commercial agar fiber hydrolyzes readily in the presence of acid.

5. Cool to 60 C., and add the white of egg beaten with the crushed shells (or, for the sake of economy, ordinary beef or sheep serum in the quantity of 25-50 c c per liter[7]).

6. Autoclave for 1 hour at 115 C

7. Filter through cotton and distribute in bottles of 200-500 c c quantities.

8. Sterilize in the customary manner.

[5] Overheating by long continued sterilization should be avoided, the medium becoming dark brown and losing considerable of its nutritive value.

[6] The inexpensive agar fiber of the present market varies considerably in quality and contains from 10-15% water. Some lots of agar will be improved by previous treatment with glacial acetic acid (0.2-0.3%) and subsequent thorough washing.

[7] We are at present engaged in developing a method of clearing agar solution with charcoal. Most of the commercial preparations are very alkaline and repeated adjustment of the reaction makes the process a very tedious one.

(*d*) *Trypsinized Digest Broth:*

1. Peptic digest prepared up to Stage 4 of the general outline is not strained or placed in the ice-chest, but is cooled to 80 C. and made faintly alkaline to litmus with twice normal KOH or twice normal sodium carbonate.

2. Cool to 37 C. and add 1% pancreatic extract (prepared according to the methods given in Plimmer's Practical Organic and Biochemistry, London, 1915, p. 405, or in the paper of Cole and Onslow, *Lancet,* 1916, 2, p. 10) or "Bacto" trypsin (marketed in sterile ampoules by the Digestive Ferment Company, Detroit).

3. Keep the mixture at 37 C. for 3-10 hours depending on the action of the trypsin and the digestion desired. Control the process by repeated tests for tryptophane.

4. When trypsinizing is sufficiently advanced, render slightly acid with glacial acetic acid and bring slowly to boiling point for 10 minutes.

5. Filter through paper, or keep in cool place over night and decant the clear liquid in the morning.

6. Add 0.2% dibasic phosphate, adjust the reaction to faintly alkaline or to the desired H-ion concentration, and treat in the manner outlined in Section "*a*," or use for the preparation of agar.

(*e*) *Sugar-Free Trypsinized Digest Broth:* This can be prepared by the following modification of the method given in Section "*d*."

1. At Stage 2 of the process, add simultaneously with the pancreatic extract or trypsin solution, 0.2% of dibasic potassium phosphate, 1% calcium carbonate and 1% of a 24-hour-old broth culture of B. saccharolyte.

2. Incubate at 37 C. for 12-18 hours and control the digestion by tryptophane tests and the removal of carbohydrates by the gas formation in fermentation tubes.

3. When the digest is sugar-free, steam for 15 minutes and use for the preparation of agar; or

4. Set to the desired reaction and steam for another 15 minutes.

5. Filter through paper; the calcium carbonate present will assist materially in obtaining a perfectly clear filtrate.

6. Distribute and sterilize in the usual manner.

II. PREPARATION OF TRYPTIC DIGEST BROTH OF HUMAN PLACENTA OR BEEF HEART

1. Prepare some fresh beef hearts by removing the fat and vessels, mince finely and weigh. Fresh, human placentas are rinsed in water and also passed through a meat-chopper.

2. To 500 gm. of the minced beef hearts or human placentas, add 1,000 gm. of tapwater.[8] Make faintly alkaline to litmus with normal KOH or Na_2CO_3, and heat slowly to 70-80 C. for 5-10 minutes.

3. Cool to 37 C. and add 1% pancreatic extract or "bacto" trypsin (details are given under Heading 1, Section "*d*"), and keep at 37 C. for 2-5 hours. Control the progress of digestion by repeated biuret and tryptophane tests. In

[8] In case distilled water is used or the tap water is poor in calcium and other minerals, calcium, sodium and magnesium chlorid, as well as phosphate, have to be added. The following amounts have proven satisfactory: Sodium chlorid 0.5%; calcium chlorid 0.01%; magnesium sulphate 0.02%, and dibasic calcium phosphate, 0.2%.

case the digestion is extended over a period of 6 hours, it is necessary to add chloroform or toluene.

4. When the process is sufficiently advanced, render slightly acid with glacial acetic acid and boil slowly for 15 minutes.

5. Either filter or decant the clear fluid, which results on placing the digest over night in a cool place.

6. Adjust the reaction, add 0.2% dibasic potassium phosphate and, if necessary, the minerals (chlorid of magnesium, sodium, etc.), in which the broth is deficient for reasons stated in Footnote 8, are added.

7. Heat for 15-30 minutes in the steamer at 100 C. and filter again, if necessary.

8. Sterilize at 100 C. on 3 consecutive days, if not to be used at once.

III. PEPTIC AND TRYPTIC DIGESTS OF WHOLE BLOOD OR BLOOD CLOTS

(*a*) *Peptic Digests:*

1. Obtain from the abattoirs in clean containers 10 liters of fresh beef blood. Decant and store the serum (which has separated on standing) in a refrigerator from 12-18 hours.

2. Weigh the blood clots and mix each 100 gm. with 1 liter of tap water.

3. Place the mixture in an enameled pot, bring slowly to a boil and under constant stirring keep it at this temperature for 5 minutes.

4. Cool to 50 C., add to each liter of the mixture 100 gm. of mixed pigs' stomach (for preparation, see the instructions given in the appendix under Method 1), transfer to glass or porcelain receptacle, and finally add 1% hydrochloric acid.

5. Digest at 50 C. for 18-24 hours and treat the resulting digest as outlined in Method 1. (Steps 3-6, and Section "*a*," steps 1-3).

6. Clear the neutralized broth or agar by adding 5-10% of the decanted beef serum, steam for 45-60 minutes.

7. Remove the flasks or bottles from the steamer and allow the clot to form a compact mass; decant or, better, centrifuge the medium to remove it.

8. Sterilize at 100 C. as customary.

(*b*) *Tryptic Digests:*

1. The preparation of the blood substratum for digestion is practically identical, as given under Section "*a*," Stages 1-4. Use, however, 500 gm. of blood clot to 1 liter of tap water.

2. Strain the fluid portion of the mixture through cheese cloth and pass the residue through a fruit press. Cool to 37 C.

3. Make the thick, brownish fluid slightly alkaline to litmus, add 1% pancreatic extract and keep at 37 C. for 5-24-48 hours.

4. The further treatment of the digest is the same as given under Heading II, Stages 4-8.

5. The neutralized broth or agar can be cleared with decanted serum, the resulting medium is excellent for primary isolation of highly parasitic organisms.